Response

Claim 201 is amended to incorporate a functional limitation wherein the claimed siRNA exhibits a reduction in the level of off-target gene silencing by both strands. Support for the amendment can be found, for example, in the published application in Example 20, paragraphs 499-501 and also at paragraphs 64-66, and 361. No new matter has been added. Claims 1-200, 202-212, 214-219, 222-223, and 227-236 are canceled. Claims 201, 213, 220-221, 224-226, 237 and 238 are pending.

The foregoing amendments are made solely to expedite prosecution of the application and are not intended to limit the scope of the invention. Further, the amendments to the claims are made without prejudice to the pending or now canceled claims or to any subject matter pursued in a related application. The Applicants reserve the right to prosecute any canceled subject matter at a later time or in a later filed divisional, continuation, or continuation-in-part application.

In view of these amendments, and further in view of arguments presented below, the Applicants respectfully request reconsideration and earnestly seek allowance.

In the Office Action mailed October 21, 2009, the Examiner found Applicants' arguments filed on August 17, 2009, with respect to the previous rejections under 35 U.S.C. § 112, 1st paragraph (new matter); and 35 U.S.C. § 103(a) over Giese et al., in view of Elbashir et al. and Vargeese et al., to be persuasive. Therefore, the previous rejections were withdrawn.

In the Office Action mailed October 21, 2009, the Examiner newly rejects claims 201, 213, 220, 221, 224-226, 237 and 238 under 35 U.S.C. § 112, 1st paragraph (new matter); and 35 U.S.C. § 103(a) over Giese et al., in view of Elbashir et al., Vargeese et al., Jackson et al. (Nature Biotechnology, 2003, pp 635-637) and Bartelmez (US 6,841,542). The Examiner also raises the issue of double patenting.

Claim Rejections - 35 USC § 112

Claims 201, 213, 220, 221, 224-226, 237, and 238 are rejected under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the written description requirement. The claim(s) allegedly contains subject matter which was not described in the specification in such a way as to

reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

Claim 201 was previously amended to require for the siRNA molecule to be "configured" for interacting with a target mRNA. However, the Examiner states that this terminology is not supported by the specification and is not defined. Therefore, for purposes of advancing prosecution, claim 201 is amended to remove the term "configured", which renders the rejection moot. Reconsideration of the rejection is respectfully requested.

Claim Rejections - 35 USC § 103

Claims 201, 213, 220, 221, 224-226, 237, and 238 have been rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Giese et al. (US 2004/0180351 A1), in view of Elbashir et al. (The EMBO Journal, 2001, Vol. 20, No. 23, pages 6877-6888), Vargeese et al. (US 2004/0110296 A1), Jackson et al. (Nature Biotechnology, 2003, pages 635- 637), and Bartelmez et al. (US 6,841,542).

With respect to Giese et al., the Examiner expounds as follows:

> Giese et al. teach siRNA molecules comprising a sense and an antisense strand, wherein the antisense region is complementary with the mRNA of a target gene and is complementary with the sense region.
>
> Giese et al. teach various combinations and patterns of modifications for siRNA duplexes. Giese et al. teach that the siRNAs can be blunt-ended or can comprise a 3'-overhang of at least one nucleotide on the sense or antisense strand. Giese et al. teach siRNA molecules fully modified with 2'-O-methyl modifications, as well as siRNA modification schematics with alternating 2'-O-methyl regions (see Figure 2, for example). Giese et al. teach an siRNA, for example, that is fully modified with 2'-O- methyl modifications with 2 nt 3'-overhangs on the sense and antisense strands (TT) (see duplex 79A79B in Figure 8, for example).
>
> Giese et al. teach that it is particularly advantageous to inactivate the sense strand of any of the RNAi forms of any of the embodiments, preferably via end modification, and more preferably a 5' end modification. Giese et al. teach that the advantage of this strategy arises from the inactivation of the sense strand which might otherwise interfere with an unrelated single-stranded RNA in the cell (see paragraphs [0103] and [0167]). Furthermore, Giese et al. teach that the 5' end of the antisense strand preferably has a free OH and that the 5' end of the sense strand is modified to inactivate the strand (see paragraph [0103] and Table 1, embodiments 7 and 8). (emphasis added by Applicant)

Giese et al. teach that a 5'-phosphate on the antisense strand is required for siRNA function, suggesting that cells check the authenticity of siRNAs through a free 5' OH which can be phosphorylated and allow only such bona fide siRNAs to direct target RNA destruction (see paragraph [0119]). (emphasis added by Applicant)

Giese et al. teach that each of the design elements may be combined (see paragraphs [0112] and [0113], for example). Giese et al. teach that in addition to the various modifications or designs of the inventive RNAi molecules, further or additional modification of the nucleotides may include the use of a phosphorothioate backbone of the RNAi molecules which may be either complete or partial in order to inhibit endonuclease function (see paragraph [0170]).

Giese et al. teach that 2'-O-alkyl modifications stabilize RNAi molecules against degradation, but to a certain degree this is counterbalanced by the effect that 2'-alkyl modifications generally result in a reduced knockdown activity. Therefore, Giese et al. offers motivation to incorporate 2' -O-alkyl modifications in specific locations, rather than to blanket the siRNA with such modifications. Giese et al. offers motivation to incorporate such modifications in a manner that is minimal enough to not reduce knockdown activity. Giese et al. teach that accordingly, the design of RNAi molecules has to balance stability against activity (see paragraph [0176]). Giese et al. teach that the most efficient molecules were modified at alternating positions of both strands. (emphasis added by Applicant)

Giese teaches incorporation of various 2'-position modifications including amino, fluoro, methoxy, alkoxy, and alkyl (see paragraph [0024]). Giese et al. teach siRNAs with various end modifications on the sense and antisense strand and particularly teach the sense strand should be modified at the 5' end to reduce off-target effects. (see paragraphs 0103 and 0173).

However, the Examiner states that Giese et al. do not teach a specific schematic wherein the first two nucleotides of the sense and antisense strands (from the 5' end) are 2'-O-alkyl modified wherein the rest of the nucleotides are 2'-OH and does not teach conjugates.

With respect to Elbashir et al., the Examiner states:

Elbashir et al., teach siRNAs, wherein each strand is 21-23 nucleotides in length and wherein at least 19 nucleotides of the sense strand are complementary to the antisense strand. The siRNAs taught by Elbashir et al. mediated RNAi via RISC. Elbashir et al. teach chemical modification with 2'-deoxy or 2'-O-methyl modifications. Elbashir et al. teach modification of 19% of the nucleotides of a duplex 21 nucleotides in length with 2'-deoxy modifications that retained activity, wherein the modifications were in the 3' terminal regions.

Elbashir et al. teaches that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA function (see page 6886, column 2); the siRNA molecules comprise ribonucleotides (see Fig. 1, for example); duplexes of 21 nt siRNAs with 2 nt 3'-overhangs were the most efficient triggers of sequence-specific mRNA degradation (see abstract, for example); and modification of the overhangs (see page

6881). Elbashir et al. teaches that 2'-deoxy substitutions help to reduce the cost of RNA synthesis and may enhance RNase resistance of siRNA duplexes (see page 6885, column 1).

With respect to Vargeese et al., the Examiner states:

> Vargeese et al. teach conjugates including cholesterol, wherein the cholesterol conjugate is for the delivery of a siRNA molecule (see abstract and paragraph [0009], for example). Vargeese et al. teach that the conjugates are used to facilitate delivery of molecules into a biological system such as a cell. Vargeese et al. teach that the conjugates can impart therapeutic activity by transferring therapeutic compounds across cellular membranes (see paragraph [0009]).

With respect to Jackson et al., the Examiner states:

> Jackson et al. teach the use of gene expression profiling to characterize the specificity of gene silencing using siRNA and discovered that both the sense and the antisense strand was responsible for off-target gene silencing (see page 636).

With respect to Bartelmez et al., the Examiner states:

> Bartelmez et al. discloses solutions to the problem of non-target binding of antisense compounds and teach the antisense strand can be modified to reduce the non-target binding (see column 14, particularly lines 50-63).

According to the Examiner, it would have been obvious to incorporate a block of 2'-O-methyl modifications at the 5' end of the sense and antisense strands, more particularly two of such modifications at the end of each strand, as well as to incorporate cholesterol and to couple the cholesterol to the 3' end of the sense or antisense strand.

The following arguments are stated by the Examiner to be in support of the obviousness rejection over the five cited references:

> The Examiner alleges it would have been obvious to one of ordinary skill in the art at the time the invention, and a matter of routine experimentation, to use the general conditions taught by Giese et al. for making 2'-modified siRNA to impart increased stability and functionality in any siRNA and to modify the 5' end of the sense and antisense strand to reduce off target effects as well as not introduce internal 2'-O-methyl groups into the siRNA, given that Giese et al. teaches that 2'-O-methyl modifications are beneficial but decrease silencing activity if incorporated at too many positions; and Elbashir et al. teaches that terminal modifications are well tolerated.

The Examiner argues that one would have been motivated to incorporate a cholesterol conjugate into the siRNA molecules of Giese et al. and would have been motivated to couple the conjugate molecule to the 3' end of the sense or antisense strand because Vargeese et al. teaches that cholesterol conjugates are used to facilitate delivery of molecules into a biological system such as a cell.

With regards to the cholesterol conjugate being coupled at the 3' end of the sense or antisense strand, the Examiner considers an element of routine optimization to determine the optimal location within the duplexes of Giese et al. Moreover, the Examiner states that Giese et al. teaches that necessity of a 5' phosphate on the antisense strand for active siRNA molecules, therefore one would have been motivated to incorporate the conjugate at the 3' end.

The Examiner alleges it would have been prima facie obvious to perform routine optimization to determine optimal location for coupling the cholesterol conjugate of Vargeese et al., as well as to incorporate the chemical modifications of Giese and Elbashir in various combinations/locations, especially within the guidelines of Giese with regards to inactivating the sense strand and maintaining an active antisense strand, as noted in In re Aller, 105 USPQ 233 at 235,

According to the Examiner, routine optimization is not considered inventive and no evidence has been presented that the particular administration ranges used were other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art.

Further, the Examiner explains that Elbashir et al. teach that full 2'-0-methyl modification of both strands of the duplex as well as either sense or antisense strand alone, abolished RNAi activity. This combined with the teachings of Giese et al. that 2'-0-methyl modifications are beneficial if incorporated in a manner that does not significantly damage RNAi activity would motivate the skilled artisan to test these modifications in particular locations, particularly in terminal nucleotides, given that Elbashir et al. teaches that terminal modifications are well tolerated. It is considered a matter of routine experimentation to determine the optimum number and placement of the 2'-modifications to see how well the modifications were tolerated with respect to ,the functionality of the dsRNA, particularly given all that was known in the prior art regarding the benefits of incorporating 2'-0 modifications to RNA.

Moreover, the Examiner argue that given that Giese et al. teach modification of siRNA to inactivate the sense strand, one would have been motivated to introduce 2'-0-alkyl groups, particularly 2-0-methyl groups at the 5' end of the sense strand to reduce off target effects and increase the specificity of the siRNA. The problem of non-target effects with antisense strands have been known in the prior art and solutions have been proposed to counteract these non-target effects. Jackson et al. discovered similar problems with antisense strands in RNAi and that when using double stranded RNA the antisense strand was also capable of non-target effects. It therefore not only would have obvious to routinely optimize the molecules for stability/activity balance, but also would have been obvious to one of ordinary skill in the art to incorporate 2'-0 modifications in the antisense strand and because Giese et al. teach modification of the sense strand at the

5' end reduced off-target effects, one of ordinary skill in the art would have looked to Giese et al. for guidance in incorporating modifications in particular nucleotides of the 5' end of the antisense strand to reduce off-target effects as well.

The Examiner states it is within the realm of routine optimization to combine the siRNA modifications of the prior art into various modification schematics to optimize the activity and stability of the molecule. It appears that the inventive feature upon which Applicants are relying is modification of the 5' end of the sense strand to inactivate the sense strand combined with modifications of the antisense strand that yield an active strand. Importantly, this feature is taught by Giese et al. Although Giese et al. does not teach the instant specific configuration, Giese teaches that it is particularly advantageous to inactivate the sense strand of any of the RNAi forms of any of the embodiments, preferably via end modification, and more preferably a 5' end modification. Giese et al. teach that the advantage of this strategy arises from the inactivation of the sense strand which might otherwise interfere with an unrelated single-stranded RNA in the cell; that the 5' end of the antisense strand preferably has a free OH and that the 5' end of the sense strand is modified to inactivate the strand. Furthermore, Giese et al. teach that a 5'-phosphate on the antisense strand is required for siRNA function, suggesting that cells check the authenticity of siRNAs through a free 5' OH which can be phosphorylated and allow only such bona fide siRNAs to direct target RNA destruction.

Therefore, the Examiner concludes that Giese teaches to inactivate the sense strand via modifying the Page 15 terminal 5'nucleotide; teaches that the antisense strand requires a 5'-phosphate for function; and teaches incorporation of the instant type of chemical modification. Although Giese does not teach to specifically modify the first two nucleotides of each strand only, it is certainly within the realm of routine optimization to do so, especially given that Giese teaches throughout the document that modifications are preferably incorporated in blocks of one or more nucleotides.

Finally, the Examiner argues that one of skill in the art would have had a reasonable expectation of success at incorporating a cholesterol conjugate into the siRNA molecules of Giese et al. and would have a reasonable expectation of success when coupling the conjugate molecule to the 3' end of the sense or antisense strand because Giese et al. teaches that a 5' phosphate on the antisense strand is necessary for activity and Vargeese et al. teaches the advantages of conjugating nucleic acids such as siRNAs to conjugates such as cholesterol. The Examiner believes that one would reasonably expect for a cholesterol conjugate to benefit the delivery of the siRNA molecules of Giese et al. given the teachings of Vargeese et al.

Furthermore, the Examiner alleges that one of skill in the art would have had a reasonable expectation of success in optimizing the siRNA molecules of Giese via terminally the first two nucleotides of 5'end of each strand with 2'-O-methyl modifications, given that this type of modification was known to enhance the stability and activity of siRNA molecules and that the modification needs to be minimized to retain activity, as taught by Giese and Elbashir. The Examiner believes that one would reasonably expect that incorporation of each of the prior art design elements (modifications) in combination within the guidance of Giese et al. regarding modification for inactivating the sense strand and maintaining an active antisense strand to result in an active siRNA molecule.

Thus, according to the Examiner, in the absence of evidence to the contrary, the invention as a whole would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made.

Examiner's Response to previous arguments considered pertinent to the instant rejection and Applicant's Comments

The Examiner complains that Applicants argued that the instant modification schematic is not a matter of routine optimization because the claimed molecules significantly reduce off target effects in comparison to other siRNAs. However, the Examiner does not find this to be an element of the instant claims. The instant claims are compound claims and do not require any reduction of off target effects.

The Applicant responds that the claims have been amended; in part to address the Examiners concerns. Claim 201 has been amended to incorporate a functional limitation; wherein the siRNA exhibits a reduction in the level of off-target gene silencing by both strands.

The Examiner states that there is ample motivation in the art to terminally modify siRNA molecules with 2'-O-methyl modifications, as explained above.

The Applicant responds that Giese teaches away from penultimate modification. At paragraph [0123], Giese states "In a preferred embodiment the second (penultimate) nucleotide at the terminus of the strand and stretch, respectively, is an unmodified nucleotide or the beginning of group of unmodified nucleotides." Giese goes on to disclose that when the penultimate 5' nucleotide on the antisense strand is 2'-O-Me modified, the resulting siRNA is non-functional. This is the very modification specified in claim 201 in combination with other modifications which exhibited good functionality in the present case. See Example 11, Figures 16A-16C and [0193]-[0194]. Similarly, at paragraph [0173] Giese makes the generalization: "[t]his leads to the conclusion that any end of the antisense strand and more particularly the 5' end of the antisense should be kept without modifications."

The Examiner notes that, regarding result-effective variables, incorporation of the instant type of chemical modification was known to achieve a recognized result, that of enhancing stability.

The Applicant responds that the functional limitation for the instant invention is not to increase stability, but rather to enhance the specificity of the functional siRNA such that the siRNA exhibits a reduction in the level of off-target gene silencing by both strands.

> The Examiner states that there was direction in the art to decrease off-target effects as well. Contrary to Applicants' assertions, the Examiner finds that a problem of stability and off-target effects had been identified in the art and incorporation of chemical modifications, and specifically 2'-O- methyl modifications, was known to be a solution.

The Applicant responds that the Examiner has failed to provide a reference which suggests that a specific pattern of 2'-O- methyl modifications was known to be a solution to the problem of off-target effects.

> The Examiner states that the specific incorporation of two 2'-O-methyl modifications at the 5'-end of each strand is not taught in the art. However, this is the element that would result from routine optimization of the teachings of the art regarding optimal placement and number of such modifications.

The Applicant responds that the Examiner has failed to provide a lead compound with which to begin routine optimization in order to retain functionality (retain silencing of the target gene) with concomitant reduction of off-target effects (reduce silencing of off-target genes).

> The Examiner believes that Applicants' previous arguments regarding off-target effects are addressed in the new rejection under 35 USC 103(a) above. Additionally, and importantly, this is not an element of the instant claims and the motivation to incorporate the instant modifications is not required to be the reduction of off-target effects.

The Applicant responds that, as stated above, the claims have been amended to incorporate a functional limitation; wherein the siRNA exhibits a reduction in the level of off-target gene silencing by both strands. Further; a motivation to reduce off-target effects is essential since no routine optimization can occur without a result effective variable, as discussed at length in the immediately prior response (p.7), and below.

> The Examiner states that the instant pattern is a combination of known design elements and the art strongly suggests avoiding extreme modification with 2'-O-methyl

modifications and to incorporate them on a smaller scale to maintain RNAi activity, thus pointing towards the instant schematic falling within the genus of routine optimization. Although Giese et al. teaches incorporation of these modifications in alternating blocks, the instant modifications are still incorporated in a block, just a single block rather than alternating; and Elbashir et al. suggests that terminal modifications are better tolerated than internal modifications.

Applicant traverses the assertion that the Giese teaching of alternating blocks of 2'-O-Me modification for the purpose of enhancing stability would lead to the claimed pattern of modification for the purpose of enhancing specificity. In addition, the Applicant asserts the teachings of Elbashir do not provide direct support for 2'-O-Me 5' terminal modification. In page 6881-2 and Figure 4, Elbashir show that substitution of both antisense and sense 2 nt 3' overhangs with 2'-deoxynucleotides had no effect on silencing of the target gene compared to the unmodified siRNA. Elbashir did not test 2'-O-Me nucleotide modification on the 2 nt 3' overhangs with; and further did not test 2'-O-Me nucleotide modification of the 2 nt on the 5' end of strands. Further, Elbashir replaced uridine residues with 2'-deoxythymidine. (Fig. 4 legend). It is also noted that Elbashir was testing for activity and not specificity.

The Examiner states that Applicants argue Giese et al. teach away from 5' antisense penultimate modifications. While Giese et al. exemplifies a specific example of a 2' -O-modification of the penultimate 5' nucleotide with abolished activity, Giese et al. also teach many other modification patterns wherein the penultimate position of the 5' end of the antisense strand comprises a 2'-O modified nucleotides. Thus, Giese et al. discloses various patterns of modifications and does not specifically teach that modification of the penultimate position of the antisense strand should always be unmodified when configuring a dsRNA for use in RNAi.

Applicants again do not agree with the assertion that the teaching of certain polynucleotide block modification patterns for the purpose of enhancing nuclease resistance (increased stability) would lead to the instantly claimed siRNA wherein the siRNA exhibits a reduction in the level of off-target gene silencing by both strands (increased specificity). This is especially in view of the fact that the paired 2'-O-methyl modification on the antisense strand was unexpectedly found to be the key for reducing off-target effects caused by both strands.

> The Examiner states that the conclusion that the 5' end of the antisense strand should be kept without modifications of Giese et al. is with regards to a specific example. This example is not identical to the instant modification schematic. Furthermore, Giese et al. exemplifies other siRNA molecules that are 5'end modified on the antisense strand and did maintain activity. Therefore, the Examiner alleges the skilled artisan would be motivated to optimize siRNA molecules via testing for optimal placement of 2'-O-methyl modifications, which would not necessarily exclude the 5'antisense end. The Examiner offers as an example, Giese et al. teach an assay wherein a preference was observed for molecules which were modified at every second nucleotide beginning with the most 5' terminal nucleotide of the antisense strand.

The Applicant responds that Giese makes the generalization: "[t]his leads to the conclusion that any end of the antisense strand and more particularly the 5' end of the antisense should be kept without modifications." [0173] Further, Applicant again asserts that the particular alternating modification pattern of Giese is not encompassed by the claimed invention.

> The Examiner states that Giese et al. teach another experiment wherein the only siRNA that was efficient at mediating RNAi was modified with 2'-O-methyl modifications at the terminal 5' and 3' nucleotides of the antisense strand (see paragraph [0193]. Therefore, the Examiner concludes that Giese et al. certainly would not be read by the skilled artisan as teaching an absolute avoidance of antisense 5'-end modification.

The Applicant responds that Giese discloses "In a preferred embodiment the second (penultimate) nucleotide at the terminus of the strand and stretch, respectively, is an unmodified nucleotide or the beginning of group of unmodified nucleotides." [123] In a specific experiment in Example 11, Figures 16A-16C and [0193]-[0194], the functionality of various siRNA with certain alternating modification patterns was tested. "Only 19 nt long siRNA duplexes either without any or with 2'-O-methyl modification on every second other nucleotide were used." [193] Giese goes on to disclose that when the penultimate 5' nucleotide on the antisense strand is 2'-O-Me modified, the resulting siRNA is generally non-functional; with a single exception. "From the different versions of molecules with modifications on every second other nucleotide only one was efficiently mediating RNAi (FIG. 16A, molecule V5). This siRNA molecule contained an antisense strand which was modified at the most terminal 5' and 3' nucleotides." [193] Thus, Giese teaches a general lack of functionality in gene knockdown when the second (penultimate) nucleotide at the terminus of the strand is modified; with rare exceptions. The Examiner's assertion that the generalization of Giese "would not be read by the

skilled artisan as teaching an absolute avoidance of antisense 5'-end modification" is not an endorsement of a lead compound.

> The Examiner further states that Giese et al. teach that molecules which contained the modifications beginning with the second nucleotide at the 5' end of the antisense strand were more stable but had a strongly reduced activity in silencing.

The Applicant agrees with the Examiner's recitation that Giese generally teaches that molecules which contained the modifications beginning with the second nucleotide at the 5' end of the antisense strand had a strongly reduced activity in silencing.

> The Examiner states that Giese et al. conclude that therefore 2' -O-methyl modifications at particularly selected positions in the siRNA duplex can increase nuclease resistance without necessarily abolishing RNAi completely (see paragraph [0191].

Applicants emphasize that "nuclease resistance" is not a functional parameter by which the claimed molecule was identified or optimized. No off-target effect is tested in Giese with respect to 2'-O-Me modified compounds. Therefore this modification does not typify a "lead compound". Further, Giese provides no motivation to one skilled in the art to start a "routine experimentation" for optimization of reduced off-target effects.

Applicant's Respectfully Traverse new Claim Rejections under 35 USC § 103

The rejection is respectfully traversed. Never-the –less, Claim 201 is amended to incorporate a functional limitation; in part to address the Examiner's concerns. The amended claims relate to a functional siRNA having a specific and defined pattern of 2'-O-alkyl modifications, a phosphate group at the 5' end of the antisense strand; and wherein the siRNA exhibits a reduction in the level of off-target gene silencing by both strands.

Unable to find the claimed siRNA in the prior art, the Examiner relies upon five pieces of art. The first, Giese et al., describes a pattern of alternating 2'-O-Me modifications on siRNA in general, but not the specific claimed pattern of modifications. The modifications of Giese do not result in active siRNA in many instances; therefore, the Examiner relies upon a total of five pieces of art describing multiple patterns of modifications. The Examiner takes the view that

essentially all modified siRNA are obvious if the modifications are to be found somewhere in the art. The previous rejection under 103 (a) over Giese, Elbashir, and Vargeese was withdrawn in light of previous arguments. In the new rejection, the Examiner again ignores the fact that this type of obviousness rejection is precisely the hindsight reconstruction analysis warned by the Federal Circuit (CAFC), and adds two new pieces of art in an attempt to overcome the previously persuasive arguments.

A Discussion of Legal Precedent applicable to the Obviousness Rejection

The Supreme Court in *KSR* clearly stated that obviousness cannot be found by merely identifying claim elements independently in the prior art. *KSR*, 127 S. Ct. 1727, 1740 ("As is clear from cases such as Adams a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art"). Accordingly, the Examiner's premise for the rejections runs directly contrary to those binding precedential cases.

As discussed in the previous office action, the Examiner has failed to establish a *prima facie* case of obviousness under *Eisai Co. v. Dr. Reddy's Lab,* 533 F.3d 1353, 1359 (Fed. Cir. 2008) (emphasis added), which explains that a finding of obviousness is appropriate only if: "(1) there is a starting reference point in the prior art from which a skilled artisan might identify a problem and pursue a potential solution; (2) there is some reason in the prior art to make particular modifications to achieve the claimed compounds; and (3) there was some reason for narrowing the prior art universe to "a finite number of identified, predictable solutions." The CAFC emphasized: "To the extent an art is unpredictable as the chemical arts often are, *KSR's* focus on these 'identified, predictable solutions' may present a difficult hurdle [when trying to establish a case of obviousness] because potential solutions are less likely to be genuinely predictable." *Id.*

(1) The current Office Action has not identified a lead compound in the primary reference Giese et al., nor in any of the secondary references Elbashir et al., Vargeese et al., Jackson et al., or Bartelmez et al.

(2) The current Office Action has not identified reasons to make specific modifications to any compound and has not shown any degree of predictability in reducing off-target effects of both the sense and antisense strand during the relevant time period. As the Examiner recognizes, the modifications taught by Giese (discussed below) are intended to inactivate the sense strand, thereby reducing the potential for off-target effects caused by the sense strand.

(3) The current Office Action has not provided any reason for narrowing the prior art universe to "a finite number of identified, predictable solutions."

The primary reference, Giese et al., provides no motivation to one skilled in the art to start a "routine experimentation" for reduced off-target effects for the antisense strand, which wasn't even recognized as a problem by the authors. Further, Giese provide no starting point (lead compound). Without a lead compound and a specific motivation to start modification of that lead compound, no routine experimentation can occur. MPEP 2144.05(II)(B), in its title, cautions: "Only Result-Effective Variables Can be Optimized." In the first sentence of that section the MPEP requires that "A particular parameter must first be recognized as a result-effective variable, *i.e.,* a variable which achieves a recognized result, before the determination of the optimum or workable ranges of said variable might be characterized as routine experimentation." Thus, in order for the doctrine to be applicable, there must have at the time been a known relationship such that the change in a variable led to a predictable result. However, the Office Action has not shown any teaching or suggestion in the cited references that correlates the reduction of off-target activity of both the sense and antisense strands with specific modifications on specific nucleotides.

In addition, from a virtually infinite number of options available for modification of siRNA compounds (numbers, positions, types etc), the instant claims are based at least in part on the unpredictable discovery that siRNA having the specific combination of elements result in functional siRNA wherein the siRNA exhibits a reduction in the level of off-target gene silencing by both strands.

Applicants assert that the obviousness rejections of the instant claims are possible only with hindsight in view of their specific combination requirements out of the infinite options and

in view of the unpredictability of the art, in other words, breaking the claims down to their component elements, searching for each element in the prior art, and putting the elements back together using only hindsight provided by the instant specification as a guide to arrive at the instant claims. See *Ortho-McNeil Pharmaceutical Inc., v. Mylan* (Fed. Cir. 2008) at 10 ("[i]n other words, Mylan's expert, Dr. Anderson, simply retraced the path of the inventor with hindsight, discounted the number and complexity of the alternatives, and concluded that the invention of topiramate was obvious. Of course this reasoning is always inappropriate for an obviousness test based on the language of title 35…").

Precedential case law requires that, particularly with respect to unpredictable fields, obviousness rejections cannot be based solely on hindsight from Applicant's claims. Aside from hindsight based on the claims themselves, the Examiner identifies nothing in the prior art that would motivate one to select the specific lead siRNA pattern of modifications from the huge number of unpredictable choices available, or to modify any lead siRNA in the very specific manner required by the claims. As above, see *KSR*, 127 S. Ct. 1727, 1740. The Giese reference provides thousands of possible modifications as options for a lead sequence; and no specific starting lead pattern of modification is identified by the Examiner, particularly in light of the claimed siRNA, wherein the siRNA exhibits a reduction in the level of off-target gene silencing by both strands. See *Takeda* at 1356-60 which reinforces a requirement to articulate reasons for selecting a lead compound where the lead was selected from only 54 choices of a prior art reference. Without a lead compound, there would be no motivation to combine Giese with any of the secondary references.

Discussion of 35 U.S.C. 103 (a) References with respect to Amended Claims.

Giese et al. disclose interfering RNA molecules, and compositions and methods using interfering RNA molecules having enhanced stability.

Giese et al. teach only two approaches of decreasing off-target effects:

(1) "First by reducing the molecular length of the siRNA molecules to the minimum requirements (18-19 nt) and thereby reducing the chance of homology to off targets." [167]

(2) "Second, by inactivation of the sense strand to prevent a unwanted RNA silencing caused by accidental complementarity of the sense strand to a unrelated target RNA(see also Example 6)" [167] "It is particularly advantageous to inactivate the sense strand of any of the RNAi forms or embodiments disclosed herein, preferably via an end modification, and more preferably a 5' end modification." [0103]

Particularly favored embodiments are found in [101], Table 1, embodiments 7 and 8. [103] Giese et al. teach or suggest no other method of inactivation of the sense strand; and no other approach to reduction of off-target effects other than end modification. Giese et al. give an example of end modification in Example 6 where "...NH_2 end modification can be used to inactivate the sense strand on the 5' and 3' end and therefore reduce off-target effects". [173].

Further, Giese expand on the results of Example 6 and teach that with respect to RNAi activity, "any end of the antisense strand, and more particularly the 5' end of the antisense should be kept without modifications." [173] (emphasis added)

This teaching is emphasized by the Examiner in the office action: "Giese et al. teach that the 5' end of the antisense strand preferably has a free OH and that the 5' end of the sense strand is modified to inactivate the strand (see paragraph [0103] and Table 1, embodiments 7 and 8)." The Examiner also cites Giese as stating that a 5' phosphate on the target complementary strand is required for siRNA functionality; however, the full citation should be read as:

> A 5'-phosphate on the target-complementary strand of the siRNA duplex is required for siRNA function, suggesting that cells check the authenticity of siRNAs through a free 5' OH which can be phosphorylated and allow only such bona fide siRNAs to direct target RNA destruction (see paragraph [0119]). (emphasis added)

Careful reading of this paragraph suggests that the 5' end of the antisense strand preferably has a free OH, in agreement with Giese at [103].

In contrast to the teachings of Giese et al., the instant application claims a functional synthetic siRNA of 18-30 base pairs; with three unique modifications including (1) a 5' phosphate antisense modification; (2) 2'-O-methylation of positions 1 and 2 of the sense strand; and (3) 2'-O-methylation of positions 1 and 2 of the antisense strand modification wherein the claimed siRNA exhibits a reduction in the level of off-target gene silencing by both strands.

Surprisingly, only the combination of all three modifications leads to drastic reduction in off-target effects that are generated by both strands. [499] to [501]

Further in contrast to Giese et al., instant Example 20 illustrates the result that inactivation of only the sense strand reduces off-target signature from the sense strand, but increases off-target signature from the antisense strand, which led the inventors to identify a modification pattern for the antisense to minimize off-target effects caused by the antisense strand. [500]

Giese et al. teach certain 2'-O-methylation patterns as being beneficial to stability; for example, fully 2'-O-Me modified with 2'-OH on each end show enhanced stability in FIG. 8 in serum. However, Giese et al. also teach that although 2'-O-Alkyl (methyl) modifications stabilize RNAi molecules, but also result in reduction of their activity as appreciated by the Examiner (page 8 office action). [74] In contrast, the molecule of amended claim 201 that has minimal off-target effects, silenced the intended target as well or better than siRNA that contained only a 5'-phosphate group on the 5'-end of the antisense strand. [501]

Therefore, Giese et al. is deficient in teaching or suggesting multiple aspects of amended claim 201, wherein the claimed siRNA exhibits a reduction in the level of off-target gene silencing by both strands. Giese et al. do not teach the claimed strand length of 18-30 bp, but suggest only 18-19 bp strand length, as discussed above, to reduce off-target effects. Giese et al. do not teach or suggest a combination of the modifications may be beneficial to decreasing off-target effects. As the Examiner acknowledges, Giese et al. do not teach a specific schematic wherein the first two nucleotides of the sense and antisense strands (from the 5' end) are 2'-O-alkyl modified wherein the rest of the nucleotides are 2'-OH and does not teach conjugates.

Particularly, Giese et al. do not teach or suggest the claimed combination of (1) a 5' phosphate antisense modification; (2) 2'-O-methylation of positions 1 and 2 of the sense strand; and (3) 2'-O-methylation of positions 1 and 2 of the antisense strand modification leads to drastic reduction in off-target effects that are generated by both strands, as discussed above. Giese et al. do not even teach or suggest anti-sense strand off-targeting, let alone any strategy to reduce antisense strand off-targeting. Giese do not provide a lead compound, wherein the siRNA exhibits a reduction in the level of off-target gene silencing by both strands. Further, nothing in

Giese provides motivation for a skilled artisan to look further into other references to make the claimed invention by combining the teachings/suggestions therein.

Even if one were motivated to go further, the deficiencies of Giese et al. are not cured by Elbashir et al. Elbashir et al. teach siRNAs, wherein each strand is 21-23 nucleotides in length and wherein at least 19 nucleotides of the sense strand are complementary to the antisense strand. Optimum strand length was disclosed wherein duplexes of 21 NT siRNAs with 2 NT 3' overhangs were the most efficient triggers of specific mRNA degradation. Elbashir et al. teach certain chemical modification of NTs including 2'-deoxy or 2'-O-methyl modifications. Elbashir et al. teaches that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA function (see page 6886, column 2); Elbashir et al. teaches that 2'-deoxy substitutions help to reduce the cost of RNA synthesis and may enhance RNase resistance of siRNA duplexes (see page 6885, column 1). Elbashir et al. are silent with regard to preparation of siRNA with reduced off-target effects.

The deficiencies of Giese et al. and Elbashir et al. are not cured by Vargeese et al. Vargeese et al. teach conjugates and compositions for cellular delivery including cholesterol, folate, galactose, galactosamine, N-acetyl galactosamine, PEG, phospholipid, peptide and human serum albumin (HSA) derived conjugates of biologically active compounds, including antibodies, antivirals, chemotherapeutics, peptides, proteins, hormones, nucleosides, nucleotides, non-nucleosides, and nucleic acids including enzymatic nucleic acids, DNAzymes, allozymes, antisense, dsRNA, siNA, siRNA, triplex oligonucleotides, 2,5-A chimeras, decoys and aptamers. Vargeese teach one siNA conjugate which comprises a branched cholesterol conjugate linked to the sense strand of the siNA. [647]. Vargeese et al. teach siNA conjugated via a cleavable linker to branched cholesterol dimers (Fig. 30, 33), and a siNA 3'-cholesterol conjugate Fig. 32. Vargeese et al. teach generally that the conjugates are used to facilitate delivery of molecules into a biological system such as a cell. Vargeese et al. teach generally that the conjugates can impart therapeutic activity by transferring therapeutic compounds across cellular membranes (see paragraph [0009]). Vargeese et al. are silent as to the effect on siRNA off-target effects of any conjugate, let alone a cholesterol conjugate.

The deficiencies of Giese et al., Elbashir et al. and Vargeese et al. are not cured by Jackson et al. Jackson et al. teach the use of gene expression profiling to characterize the

specificity of gene silencing using siRNA by performing single nucleotide substitutions of eight different siRNA duplexes targeted to the MAPK14. It was deduced that as few as 11 contiguous nucleotides of sequence identity are sufficient to direct silencing of nontargeted transcripts; however, the authors were unable to identify patterns that could predict off-target activity of siRNAs. P.636, Col. 2. Jackson teaches that to reduce off-target effects, the nucleotide sequence of an siRNA should be selected carefully. There is no teaching or suggestion of any modifications or conjugates to reduce off-target effects.

The deficiencies of Giese et al., Elbashir et al. and Vargeese et al. and Jackson et al. are not cured by Bartelmez et al., US 6,841,542. Bartelmez et al. suggest candidate single stranded antisense oligomers, not the double stranded siRNAs, be evaluated for cellular toxicity (Col 14, ln 48-52). The antisense oligonucleotides and siRNAs do not mediate their biological effects in the same manner thus specificity of binding of candidate antisense oligomers studied by Bartelmez may have little bearing on the claimed siRNAs. Bartelmez et al. provide no teaching or suggestion which contributes to any element of the claimed functional siRNAs.

In summary, the combined teaching of Giese et al., Elbashir et al., Vargeese et al., Jackson et al. and Bartelmez et al. neither teach nor suggest the siRNA of amended claim 201: a functional synthetic siRNA of 18-30 base pairs; with three specific modifications including (1) a 5' phosphate antisense modification; (2) 2'-O-methylation of positions 1 and 2 of the sense strand; and (3) 2'-O-methylation of positions 1 and 2 of the antisense strand modification wherein the claimed siRNA exhibits a reduction in the level of off-target gene silencing by both strands. Dependent claims 213 add the limitation wherein at least one phosphorothioate internucleotide linkage. Dependent claim 220 adds the limitation of a 3' overhang of 1-5 nucleotides on at least one of the sense or antisense strand. Dependent claim 221 adds the limitation wherein the 3' overhang has at least one phosphorothioate internucleotide linkage or at least one methylphosphonate internucleotide linkage. Dependent claims 225 and 226 provide further limitations, respectively, of at least one conjugate molecule coupled to the sense or antisense strand; wherein the conjugate is cholesterol; and wherein the conjugate molecule is coupled to the 3' end of the sense strand. Dependent claims 237 and 238, respectively, add limitations wherein at least one of the 2'-O-alkyl modifications is 2'-O-methyl; and wherein each of the 2'-O-alkyl modifications is 2'-O-alkyl.

The Examiner states that "the specific incorporation of two 2'-O-methyl modifications at the 5'-end of each strand is not taught in the art. However, this is the element that would result from routine experimentation of the teachings of the art regarding optimal placement and number of such modifications". (page 17) The Applicant respectfully disagrees since 2'-O-methyl modifications in another pattern were merely recognized to enhance stability; none of the five cited references speaks to the effect of 2'-O-alkylation on reduction of off-target effects. Therefore, the limited teaching and suggestion provided by the cited art places amended claim 201 and claims dependent thereupon far outside of the realm of routine experimentation and reasonable expectation of success.

As discussed above, surprisingly, only the combination of all three modifications of amended claim 201 leads to drastic reduction in off-target effects that are generated by both strands. [499] to [501] The uniquely modified molecule of amended claim 201 has not only minimal off-target effects, but silenced the intended target as well or better than siRNA that contained only a 5'—phosphate group on the 5'-end of the antisense strand. [501]

The Examiner is respectfully requested to reconsider the rejection in light of the amendments and discussion.

Double Patenting

Claims 201, 213, 220, 221, 224-226, 237, and 238 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-10 of U.S. Patent No. 7,595,387. The Examiner asserts that although the conflicting claims are not identical, they are not patentably distinct from each other because the instant claims and the claims of the patent are drawn to patently indistinguishable subject matter.

In the interest of advancing prosecution, a Terminal Disclaimer over U.S. Patent No. 7,595,387 is filed herewith. Therefore, withdrawal of this rejection is respectfully requested.

Conclusion

This Amendment fully responds to the Office Action mailed on October 21, 2009. Still, the Office Action may contain arguments and rejections that are not directly addressed by this Amendment because they are rendered moot in light of the preceding arguments in favor of patentability. Hence, failure of this Amendment to directly address an argument raised in the

Office Action should not be taken as an indication or admission that the Applicants believe the argument has merit. Furthermore, the claims of the present application may include other elements, not discussed in this Amendment, which are not shown, taught, or otherwise suggested by the art of record. Accordingly, the preceding arguments in favor of patentability are advanced without prejudice to other bases of patentability.

Applicants respectfully submit that the present application is in condition for allowance and solicit a notice to the effect.

The undersigned wishes to mention that the attorney of record for this case has changed, from Steven N. Hird, attorney # 51,112, to John E. Burke, attorney # 35,836; Mr. Burke is also an attorney at Merchant & Gould.

This constitutes a petition for an extension of time if one is not specifically requested. Payment of appropriate fees by credit card accompanies this filing; however, if any additional fees are due, the Director is authorized to deduct said fees from Deposit Account # 13-2725.

Respectfully submitted,

MERCHANT & GOULD

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